

Letter to the Editor

Autoimmune mechanisms in chronic idiopathic urticaria

To the Editor:

Chronic idiopathic urticaria (CIU) is defined as repeated episodes of hives occurring over a period of 6 or more weeks without an identifiable cause.¹ CIU is a common disorder with a prevalence of 0.5% in the general population.² In the 1980s, the presence of a serum factor that induced a wheal and flare response following intradermal injection of autologous serum was reported³ and has since been regarded as a helpful surrogate for autoimmunity in CIU.¹ IgG autoantibodies directed against the α subunit of the high-affinity IgE receptor (Fc ϵ RI) are thought to be the primary autoimmune mechanism of CIU, and these are reportedly present in 30% to 60% of the patients with CIU.⁴⁻⁷

We have developed an immortalized human mast cell line (LUVA) displaying Fc ϵ RI that both degranulates and releases arachidonic acid-metabolites in an Fc ϵ RI-dependent fashion.⁸ These cells express high levels of chymase and tryptase (CT)-positive and therefore display characteristics of connective tissue mast cells. We investigated mast cell prostaglandin D₂ (PgD₂) production and Ca²⁺ influxes by LUVA cells in response to incubation with sera derived from patients with CIU.

Patients with CIU and matched control subjects were recruited under a protocol approved by our human investigations committee.⁹ Autologous serum skin tests were performed by using standard methodology.¹ Serum (diluted 1:1) was incubated with LUVA cells (5×10^4) at 37°C for 30 minutes, and activation was determined via enzyme immunoassay as PgD₂ secretion (sensitivity >55 pg/mL; Cayman Chemical Co, Ann Arbor, Mich). To properly assess LUVA activation, we also measured PgD₂ concentrations in comparably diluted serum samples and subtracted this from the LUVA supernatant values. Additional PgD₂ assays were performed after incubating LUVA cells overnight with monoclonal IgE (100 ng/mL; Millipore, Temecula, Calif) to upregulate Fc ϵ RI expression.⁸ As an alternative measure of mast cell activation by CIU serum, we investigated the induction of Ca²⁺ fluxes by using the Fluo-4NW calcium assay kit (Molecular Devices, Sunnyvale, Calif). Briefly, loading buffer with 5 mM probenecid (Sigma, St Louis, Mo) was added to each well and incubated at 37°C for 30 minutes. Cells were stimulated with CIU serum and cytoplasmic [Ca²⁺]_i was determined at an extinction wavelength of 494 nm and an emission wavelength of 516 nm by using a Flexstation spectrophotometer (Molecular Devices). ATP (300 mM) and monoclonal anti-Fc ϵ RI antibodies (Upstate, Lake Placid, NY) were used as positive controls, and data are expressed as arbitrary relative fluorescence units. To evaluate the role of Fc ϵ RI-dependent and independent pathways in cellular activation,¹⁰⁻¹⁴ LUVA cells were incubated with the syk inhibitor ER27319 (10-60 μ M, Tocris, Ellisville, Mo) or pertussis toxin (100 ng, Tocris) for 2 hours at 37°C. Unless otherwise specified, data are expressed as the mean $\pm$ SD and the differences analyzed by paired *t* test.

Fourteen patients with CIU were enrolled in the study. Characteristics of patients enrolled are presented in this article's Online Repository at www.jacionline.org (Tables E1 and E2). Our CIU population was predominantly composed of women with an

average age of 40 ± 14 years, similar to what other investigators have found.^{15,16} In addition, the majority of our patients were variably noted to have well-recognized markers of autoimmunity in CIU, including anti-Fc ϵ RI antibodies (9 of 14), positive autologous serum skin test results (9 of 14), and/or antithyroid antibodies (6 of 14).

As the most prominent activation pathway triggered by LUVA mast cells,⁸ we initially focused on PgD₂ secretion. We observed increased *de novo* PgD₂ secretion after the incubation of CIU-derived serum with LUVA cells in comparison to control serum (363.3 ± 125.9 pg/mL vs 243.5 ± 94.18 pg/mL; $P < .01$; Fig 1). PgD₂ secretion was modestly (18%) but consistently (14 of 14 subjects) increased after LUVA cells were incubated overnight with IgE (768.0 ± 177.3 pg/mL to 909.0 ± 203.1 pg/mL; $P < .001$) (see Fig E1 in this article's Online Repository at www.jacionline.org). To dissect the role of Fc ϵ RI-dependent and -independent pathways in CIU, PgD₂ secretion using serum samples obtained from the 6 subjects displaying the highest induction was measured after exposing LUVA cells to either a syk inhibitor or pertussis toxin, as well as after the overnight incubation of serum with recombinant human Fc ϵ RI as described.⁹ Preincubation with the syk inhibitor decreased PgD₂ production in all 6 subjects (mean percent decrease was $27.3\% \pm 4.7\%$; $P < .05$) (Fig 2). Similarly, partial inhibition of PgD₂ secretion was observed with pertussis toxin ($18.5\% \pm 3.9\%$ decrease; $P < .05$). No synergy was observed when the syk inhibitor and pertussis toxin were combined ($30\% \pm 8.17\%$ decrease). Better inhibition of activation was observed after overnight "capture" of anti-Fc ϵ RI antibodies with excess recombinant human Fc ϵ RI ($53\% \pm 28\%$ [$n = 7$]; data not shown). We also established the ability of CIU serum to induce Ca²⁺ fluxes (see Fig E2 in this article's Online Repository www.jacionline.org). Induction of Ca²⁺ influxes was consistently observed across the entire cohort of subjects ($P < .0001$). As with the PgD₂ data, pretreatment of LUVA cells with the syk inhibitor produced consistent but incomplete inhibition of Ca²⁺ influx, comparable to the extent of inhibition observed by using anti-Fc ϵ RI antibodies (Fig E2).

Previously, basophil activation tests have been considered the benchmark diagnostic test to identify autoimmune causes of CIU.¹⁷⁻¹⁹ However, this approach is limited by the inherent variability of basophils, which requires rigorous screening of basophils from numerous donors to identify "releaser" basophils capable of Fc ϵ RI-dependent activation, and even among donors displaying this phenotype, there is day-to-day variability in this capacity.²⁰ Stringent screening is also required to ensure against high spontaneous secretion of histamine. While basophils provide useful diagnostic information, CIU is a disease targeting cutaneous, largely connective tissue, mast cells. As basophils are not considered the primary cellular target of CIU, a mast cell model would be the more ideal framework to study CIU pathophysiology. The concept that CIU involves autoimmune mechanisms that include anti-Fc ϵ RI antibodies is strongly supported by our current studies. A putative contributing role for anti-Fc ϵ RI antibodies is supported by the increase in PgD₂ secretion after overnight incubation with IgE, an intervention that increases cell-surface expression of these receptors and also as the inhibition of secretion after overnight "capture" of putative anti-Fc ϵ RI antibodies with excess recombinant Fc ϵ RI

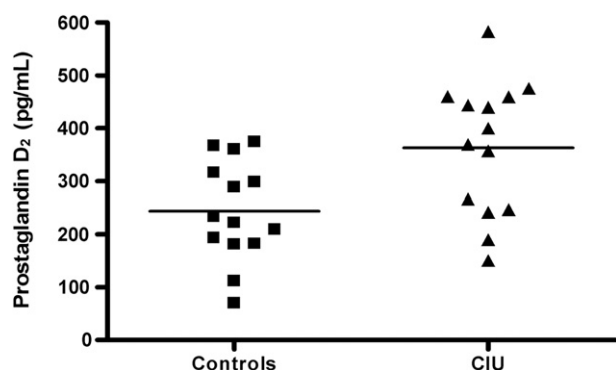


FIG 1. PgD₂ Production after incubation with control- and CIU-derived serum. Serum was incubated with LUVA cells at 37°C for 30 minutes and secreted PgD₂ assayed by enzyme immunoassay. Data represent net production of PgD₂ after subtracting out PgD₂ present in the applied serum sample ($P < .01$).

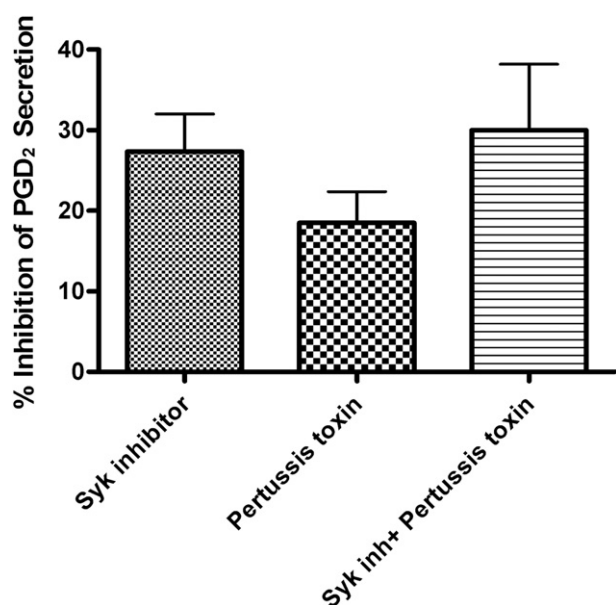


FIG 2. Inhibition of PgD₂ secretion by Syk inhibitor and pertussis toxin. LUVA mast cells were incubated with the syk inhibitor ER27319 (60 μ M) or pertussis toxin (100 ng) for 2 hours at 37°C. LUVA cells were subsequently challenged with CIU patient sera and PgD₂ secretion determined.

protein.^{8,21} It should be noted, however, that the upregulation of PgD₂ secretion induced by IgE incubation cannot categorically distinguish Fc ϵ RI-dependent effects from nonspecific priming of the LUVA cells. In further support of a role for Fc ϵ RI-mediated cell activation, application of a syk inhibitor significantly reduced PgD₂ production and Ca²⁺ fluxes. The extent of this inhibition of Ca²⁺ fluxes was comparable to that produced when cells were stimulated by anti-Fc ϵ RI antibodies. Of note, however, pertussis toxin also reduced PgD₂ production, presumably reflecting additional complementary or collateral activation pathways.¹¹ This mast cell line may also prove useful in dissecting additional mast cell-targeting autoantibodies or other complementary pathways such as those mediated by complement, bradykinin, or the clotting cascade that might contribute to the development of CIU.

In summary, we utilized a unique connective tissue mast cell line to study the effects of CIU patient sera on activation, demonstrating PgD₂ secretion and Ca²⁺ influxes. Upregulation after overnight incubation with IgE and inhibition of PgD₂ secretion and Ca²⁺ fluxes after preincubation with a syk inhibitor or recombinant human Fc ϵ RI points to a role for Fc ϵ RI-dependent autoimmune mechanisms, although additional complementary pathways appear to be involved. Future studies will be required to determine the relative validity of diagnostic testing for autoimmune mechanisms in CIU using basophil activation as opposed to assays based on what is arguably the more relevant cellular target, the connective tissue mast cell.

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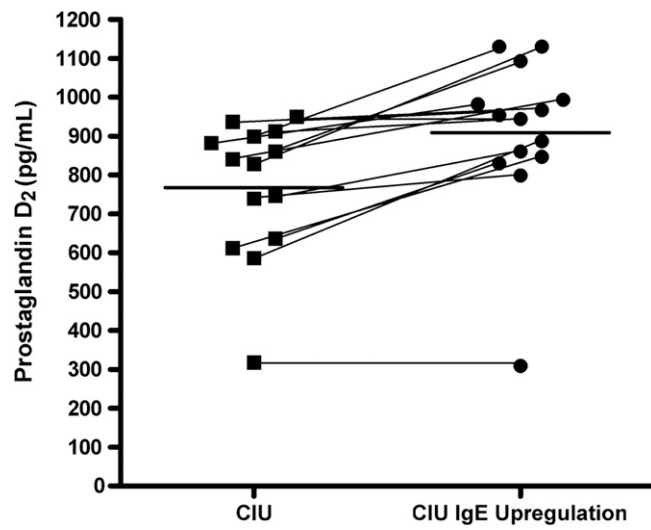


FIG E1. Upregulation of PgD₂ secretion after incubation with IgE. PgD₂ assays were repeated after incubating LUVA cells with monoclonal IgE (100 ng/mL) overnight to upregulate FcεRI expression (* $P < .0001$).

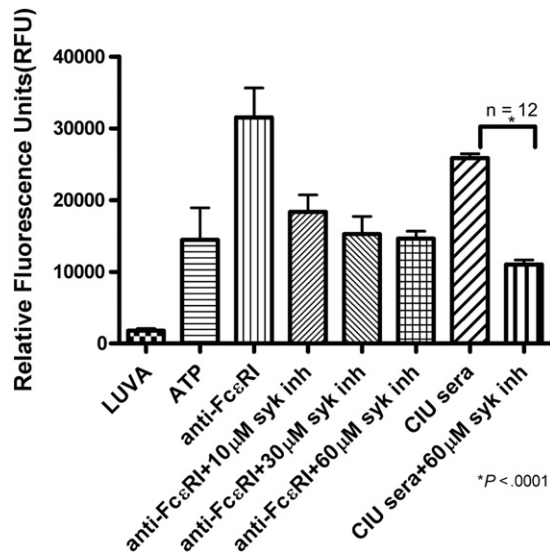


FIG E2. Induction of Ca^{2+} fluxes after incubation with CIU serum. Mast cell activation by CIU-derived serum was determined by using the Fluo-4NW calcium assay as described in the text. ATP (300 mM) and monoclonal anti-FcεRI antibodies were used as positive controls. Varying concentrations of syk inhibitor were utilized to determine optimal concentration. Each condition was performed in triplicate, and the average of the 3 measurements is presented. Data are expressed as arbitrary relative fluorescence units (RFUs). *inh*, Inhibitor.

TABLE E1. Characteristics of CIU population

Patient	Age (y)	Sex	ASST*	Anti-FcεRI antibodies†	Thyroid antibodies‡
1	61	F	Positive	Positive	Negative
2	56	F	Negative	Positive	Positive
3	28	F	Positive	Positive	Positive
4	65	M	Positive	Positive	Positive
5	35	F	Positive	Positive	Negative
6	47	M	Negative	Positive	Negative
7	61	F	Negative	Negative	Positive
8	46	F	Positive	Negative	Negative
9	21	F	Positive	Negative	Positive
10	29	M	Negative	Negative	Negative
11	40	F	Positive	Positive	Positive
12	27	F	Positive	Positive	Negative
13	27	F	Positive	Positive	Negative
14	37	F	Negative	Negative	Negative

ASST, Autologous serum skin test; *F*, female; *M*, male.

*Positive ASST result defined as wheal more than 5 mm in diameter more than saline controls.

†Positive anti-FcεRI titer defined as more than 3.9 μg/mL.⁹

‡Positive thyroid antibodies defined as a positive thyroglobulin and/or thyroid peroxidase antibody titer.

TABLE E2. Summary of characteristics of the CIU population

Characteristic	n = 14
Age (y), mean $\pm$ SD	41 $\pm$ 14
Sex (number of females)	11/14
ASST (number positive)*	9/14
Anti-Fc ϵ RI antibodies (number positive)†	9/14
Thyroid antibodies (number positive)‡	6/14

ASST, Autologous serum skin test; EIA, enzyme immunoassay.

*Positive ASST result defined as wheal more than 5 mm in diameter more than saline controls.

†Positive anti-Fc ϵ RI titer defined as more than 3.9 μ g/mL via EIA as described.⁹

‡Positive thyroid antibodies defined as a positive thyroglobulin and/or thyroid peroxidase antibody titer.